MYCOTAXON

ISSN (print) 0093-4666 (online) 2154-8889 Mycotaxon, Ltd. ©2020

October–December 2020—Volume 135, pp. 877–883

https://doi.org/10.5248/135.877

Jahnoporus oreinus, a rare fungus found in high-altitude moist temperate forests of Pakistan

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Abstract—*Jahnoporus oreinus* was collected on a buried decomposing roots of spruce during an excursion in the high altitudinal moist temperate forests in the Gabin Jabba valley of district Swat, Khyber Pakhtunkhwa, Pakistan. This species, previously reported from the Russian Far East, represents a new record for the Pakistani mycoflora. This is the first report of the genus *Jahnoporus* in Pakistan.

Key words—*Dacryobolaceae,* ITS, new record, phylogenetic analysis, *Picea*

Introduction

Swat is a floristically rich district in Pakistan's Khyber Pakhtunkhwa province, located at 34°34′–35°55′N 72°08′–72°50′E. Swat borders the Chitral and Ghizar districts in the north, Buner district and Malakand agency in the south, the Shangla and Indus Kohistan districts in the east, and the Dir district in the west. The region is mountainous, with elevations 970–2300 m a.s.l. (Kiran & al. 2017). Climatically, the district comprises dry and moist temperate regions, with lush green forests that are home to many important medicinal and aromatic plants. The forest floors are rich in humus and support a huge diversity of macrofungi. However, compared to plants, fungal research is still in its infancy in Swat, with most of the area under- or unexplored. Therefore the Centre for Plant Sciences and Biodiversity (CPS & B) of the University of Swat initiated a project with the goal of investigating fungal diversity in the under-explored areas of the district.

The present paper, which is part of this project, reports on *Jahnoporus oreinus,* previously unrecorded from Pakistan. This species, first described from eastern Russia by Spirin & al. (2015), is regarded as a strong indicator of pristine *Picea*-dominated forests. With no previous records for the country, *Jahnoporus* (*Dacryobolaceae*) can be regarded as rare in Pakistan. This small genus of wood inhabiting fungi is represented by four species: *J. brachiatus* Spirin & al., *J. hirtus* (Cooke) Nuss, *J. oreinus,* and *J. pekingensis* (J.D. Zhao & L.W. Xu) Y.C. Dai (Index Fungorum http://indexfungorum.org/names/names.asp).

Materials & methods

Collection and morphological examination

The collection was made in the Gabin Jabba valley of District Swat during the summer of 2017. The green valley, politically a part of Swat's Matta tehsil, is characterized by moist temperate vegetation with *Picea smithiana* and *Abies pindrow* the dominant tree species at high altitudes and *Pinus wallichiana* in the foothills (Jabeen & al. 2019). Thick forests of *Quercus semecarpifolia* Sm. are also present in the upper tree limit.

The basidiomata were collected using standard procedures and photographed in their natural habitat. Important macro-morphological characters were noted in a field notebook. Colors were coded following to the Munsell (1975) soil color chart. A fan heater with temperature adjusted at 45–50°C was used for drying the specimens. Properly dried specimens were deposited in the Herbarium, University of Swat, Kanju Township, Pakistan (SWAT).

Microscopic characters are based on free hand sections from dried herbarium specimens mounted in 5% (w/v) aqueous potassium hydroxide (KOH) solution. Aqueous Congo red (1%; w/v) was used for contrast. Tissues were examined and photographed using a BOECO BM120 compound microscope fitted with a MVV 3000 microscopic camera. Microstructures were measured with calibrated Piximètre software v. 5.9; Q = range of length/width (L/W) ratio of all measured basidiospores, Qe = the average L/W ratio of basidiospores, and Me = average L × W of all measured basidiospores.

DNA extraction, PCR, and sequence analysis

DNA from dried herbarium specimens was extracted using the CTAB method (Gardes & Bruns 1993). The internal transcribed spacer (ITS) region along with the central 5.8S region was amplified using the ITS1F (Gardes & Bruns 1993) and ITS4 primer pair (White & al. 1990).

For the polymerase chain reaction (PCR), we followed the methods mentioned in Khan & al. (2017). Two nucleotide sequences were generated from the two different pilei. Both the forward and reverse primer reads were assembled in BioEdit v.7.2.5 (Hall 1999) and consensus sequences were generated. The two consensus sequences

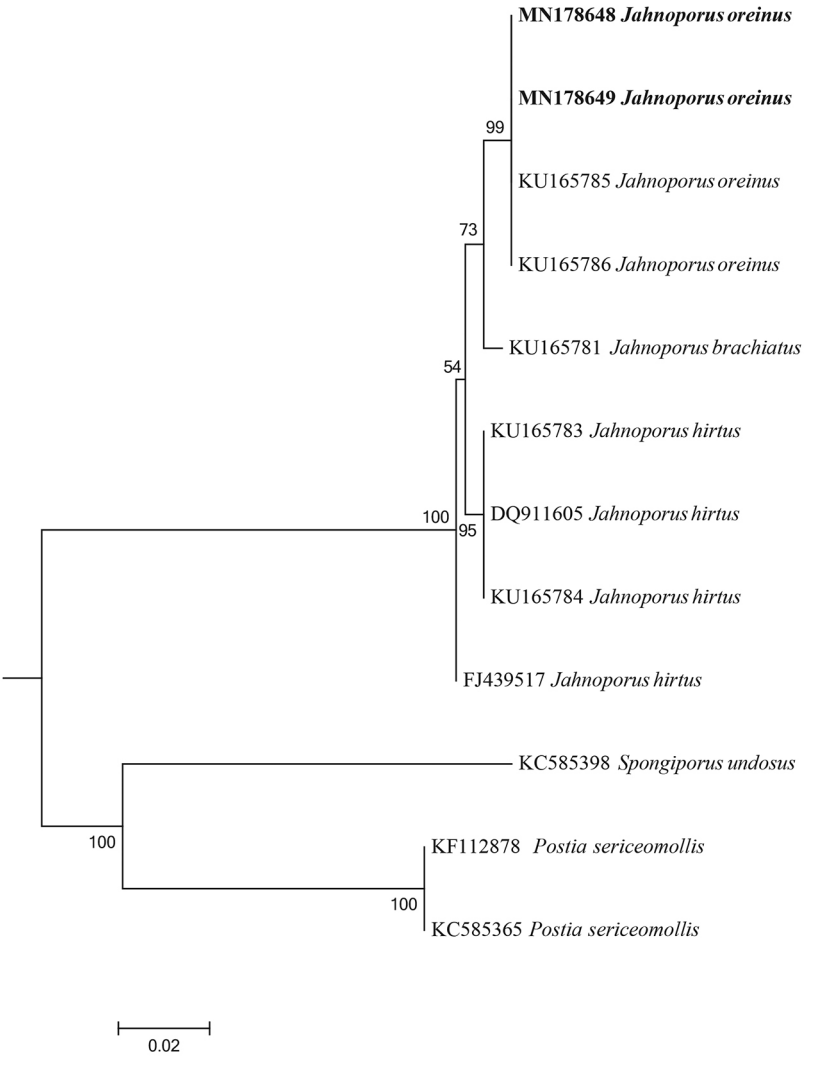

Fig. 1. nrITS based maximum likelihood analysis of *Jahnoporus oreinus* and related taxa. Pakistani collection is presented in bold. Bootstrap values ≥50% are given above nodes.

BLAST searched and closely matching sequences and from published literature (Spirin & al. 2015) were downloaded for phylogenetic analysis. *Postia sericeomollis* (Romell) Jülich (KC585365; KF112878) and *Spongiporus undosus* (Peck) A. David

(KC585398) were selected as outgroup taxa. For phylogenetic inference, Maximum Likelihood (ML) analysis was performed using MEGA6 software with 1000 bootstrap replicates (Tamura & al. 2013).

Phylogenetic analysis

Two new sequences (630 bp each) were generated from two different pilei of the same collection (GJ-1513). During BLAST search, the GJ-1513 sequences showed 99.65 % similarity (different in ≤4 bp) with *Jahnoporus oreinus* (KU165786; KU165785) and 97 % similarity with *J. brachiatus* (KU165781) and *J. hirtus* (KU165782; KU165783; KU165784).

The 12 nucleotide sequences used in the phylogenetic analysis comprised four in-group and two outgroup taxa. There were a total of 571 sites in the aligned dataset, of which 176 were variable and 137 were parsimony informative. Maximum likelihood analysis clustered the ITS sequences in four clades (Fig. 1), which agree with the findings of Spirin & al. (2015). The Pakistani collection clustered with the Russian collections of *J. oreinus,* confirming its identity.

Taxonomy

Jahnoporus oreinus Spirin, Vlasák & Miettinen, Cryptog. Mycol. 36: 414 (2015) Fig. 2

Fruiting body 120 mm wide, annual, fleshy when fresh, hard when mature or dry, several fused pilei arising from a common stipe. Pileus 30–50 mm across, semi-circular to oval in shape, pale greyish-brown (7.5 YR 8/4) with a dark brownish center (5YR 3/4); surface dry, shiny, bumpy, hispid; pileus margin sharp and wavy, slightly incurved in some areas. Pore surface slightly decurrent, cream-colored, drying brownish orange (5YR 5/8); pores mostly angular, some irregular, 2–3 per mm; tubes 2–3 mm deep at the center, 1mm at the edge, concolorous with the pore surface. Stipe 40–50 × 8–12 mm, eccentric, hispid, hairs denser and longer than on pileus, yellowish brown (7.5YR 6/8) upward, strong brown (7.5YR 3/6), interior solid, white. Odor and taste mild.

Basidiospores (13–)13.5–15.9(–16.2) × (4.1–)4.2–5.4(–5.6) µm, Q = (2.7–)3–3.2(–3.3), Me = 15.1 × 4.8 µm, Qe = 3.1, fusoid in face view, navicular in side view, smooth, guttulate. Basidia (35–)40–47(–50) × (9–)10.5–11.5(–12) µm, clavate to narrowly clavate, mostly 2-sterigmate, rarely with 4-sterigmata; thin-walled, hyaline in KOH, with oily droplet, clamped at base; sterigmata 6–8 µm long. Cystidioles (32–)35–43.5(–45) × (5–)5.3–7.5(–8) µm, narrowly lageniform to bottle-shaped, thin- to thick-walled. Pileal Hairs and Upper

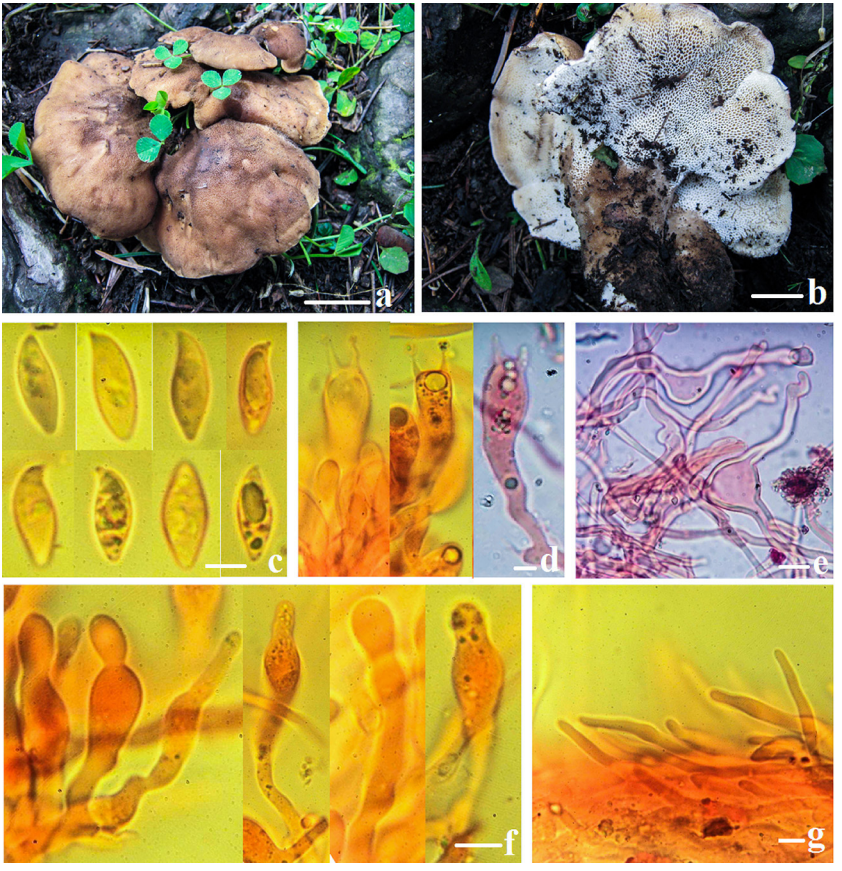

Fig. 2. *Jahnoporus oreinus* (SWAT001350). a. Basidiomata in natural habitat; b. Pore surface; c. Basidiospores; d. Basidia; e. Contextual hyphae; f. Cystidioles; g. Pileipellis hyphae. Scale bars: a, b = 10 mm; c–g = 5 μm.

Surface Hyphae parallel, thin walled, 3–5 μm in diameter. Trama Hyphae elongated, septate, 3–4 μm in diameter. Contextual Hyphae parallel, thick-walled, 3–5 μm in diam., mixed with gloeocystidia; gloeocystidia 70–100 × 10–15 μm, thick walled (≤2μm), irregular. Hyphal System monomitic, clamp connections present.

Material examined: **PAKISTAN**, **Khyber Pakhtunkhwa**, **District Swat**, Matta tehsil, Gabin Jabba valley, 2450 m a.s.l., on buried decomposing roots of *Picea smithiana* (Wall.) Boiss., 30 Aug 2017, Junaid Khan GJ-1513 (SWAT001350; GenBank MN178648, MN178649).

Discussion

Jahnoporus oreinus, a strong indicator species of the old growth spruce forest, is reported as a new record for Pakistani mycoflora. So far, this species is reported from three distant localities in far eastern Russia, with the new collection apparently the fourth record of *J. oreinus* from around the globe. The Pakistani material agrees morphologically with the original description by Spirin & al. (2015) with some differences. Most importantly, the stipe of the Pakistani collection is hirsute and covered by denser and longer hairs compared with the pileus, which differs from the smooth to rarely hairy stipe of the Russian collections. Secondly, the 2-spored basidia in the Pakistani collection are not reported in the original description. Our collection also has very frequent narrowly lageniform to bottle-shaped cystidioles contrasting with the Russian collection where these cystidioles are rare.

Despite the morphological differences between the Pakistani and Russian collections, their ITS sequences were closely similar (differing only by ≤4 bp) and clustered together in molecular phylogenetic analysis (Fig. 1), thus supporting the identification of the Pakistani collection as *J. oreinus*.

Jahnoporus brachaitus, a species reported from East Asia and the Russian Far East, can be distinguished from *J. oreinus* by its growth in lowland mixed forests, pale grey to pale ochraceous pileus, and narrowly fusoid basidiospores (Spirin & al. 2015). *Jahnoporus hirtus*, the type species of the genus, is separated by its larger (≤150 mm diam.), brownish grey to reddish brown pileus, and smaller (10.4–15.4 × 4.0–5.8 µm) basidiospores (Jahn 1973, Spirin & al. 2015). *Jahnoporus pekingensis*, described from northeast China, differs from *J. oreinus* by its larger (≤180 mm diam.) pilei and considerably smaller (9–11.2 × 2.5–3.5 µm) basidiospores (Zhao & al. 1982, Dai 2003).

Acknowledgements

Our sincere thanks to Dr. Viacheslav Spirin (Botany Unit—Mycology, Finnish Museum of Natural History, University of Helsinki, Finland) and Dr. Najam-ul-Sahar Afshan (Center for Undergraduate studies, University of the Punjab, Lahore, Pakistan) for reviewing this manuscript. We are grateful to Dr. Else Vellinga (Berkeley, CA USA) for her corrections and suggestions during pre-editorial review.

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